

Amendments to the Specification:

Please replace the paragraph at page 11, lines 22-23, with the following amended paragraph:

Figure 3C. Amino Acid Sequences of the Constant Regions of the Modified OST577-IgG1 in Comparison with the Unmodified OST577-IgG1 (SEQ ID NO: 3). The amino acid sequences of the constant region of the heavy chain of the modified IgG1 having the amino acid substitution of T250D (SEQ ID NO: 67), T250E (SEQ ID NO: 68), T250Q (SEQ ID NO: 69), M428F (SEQ ID NO: 70), M428L (SEQ ID NO: 71), T250E/M428F (SEQ ID NO: 75), and T250Q/M428L (SEQ ID NO: 76) are also shown.

Please replace the paragraph at page 12, lines 3-4, with the following amended paragraph:

Figure 3E. Amino Acid Sequences of the Constant Regions of the Modified Hu1D10-IgG2M3 in Comparison with the Unmodified Hu1D10-IgG2M3. The IgG2M3 sequence is represented by SEQ ID NO:2. M428L is represented by SEQ ID NO:57; T250Q/M428L is represented by SEQ ID NO: 74.

Please replace the paragraph at page 12, lines 5-6, with the following amended paragraph:

Figure 3F. Amino Acid Sequences of the Constant Regions of the Modified Hu1D10-IgG1 in Comparison with the Unmodified Hu1D10-IgG1. The IgG1 sequence is represented by SEQ ID NO:3. M428L is represented by SEQ ID NO:71; T250Q/M428L is represented by SEQ ID NO: 76.

Please replace the paragraph at page 12, lines 16-17, with the following amended paragraph:

Figure 3H. Amino Acid Sequences of the Constant Regions of the M428L Hu1D10-IgG3 Mutant (SEQ ID NO: 115) in Comparison with the Unmodified Hu1D10-IgG3 (SEQ ID NO:113).

Please replace the paragraph at page 12, lines 18-19, with the following amended paragraph:

Figure 3I. Amino Acid Sequences of the Constant Regions of the M428L Hu1D10-IgG4 (SEQ ID NO:116) and T250Q/M428L Hu1D10-IgG4 (SEQ ID NO:117) Mutants in Comparison with the Unmodified Hu1D10-IgG4 (SEQ ID NO:114).

Please replace the paragraph at page 15, lines 15-17, with the following amended paragraph:

Figure 17A. Binding Assay of Hu1D10-IgG2M3 Wild-Type and Mutant Antibodies to HLA-DR β Chain Allele. The binding of the wild-type or mutant Hu1D10-IgG2M3 antibodies to Raji cells was analyzed in a FACSTM binding assay, as described in Example 8.

Please replace the paragraph at page 15, lines 18-20, with the following amended paragraph:

Figure 17B. Binding Assay of Hu1D10-IgG1 Wild-Type and Mutant Antibodies to HLA-DR β Chain Allele. The binding of the wild-type or mutant Hu1D10-IgG1 antibodies to Raji cells was analyzed in a FACSTM binding assay, as described in Example 8.

Please replace the paragraph at page 65, lines 4-24, with the following amended paragraph:

NS0 cells were stably transfected with pDL208. Approximately 1×10^7 cells were washed once and resuspended in 1 ml of plain DMEM, transferred to a Gene PulserTM Cuvette (Bio-Rad[®] Laboratories), and incubated on ice for 10 minutes. Forty μ g of plasmid pDL208 was

linearized with FspI and gently mixed with the cells on ice, then the cells were electroporated by pulsing twice using a Gene Pulser™ II (Bio-Rad® Laboratories) set at 1.5 kV, 3 µF, and returned to ice for 10 minutes. The cells were diluted in 20 ml of DMEM, 10% FBS, and plated in two 96-well plates at 100 µl/well. The medium was replaced after 48 hours with MPA selection medium. Mycophenolic acid-resistant NS0 transfectants from wells apparently containing single colonies were expanded in MPA selection medium and screened after about 3 weeks by FACSTM. Approximately 1.5×10^5 cells/test were incubated in 100 µl of FACS Staining Buffer (FSB) (PBS, 1% FBS, 0.1% NaN₃) containing 10 µg/ml of biotinylated mouse anti-human β 2-microglobulin antibody (Chromaprobe, Inc., Aptos, Calif.) for 1 hour on ice. The cells were washed once with 4 ml of FSB, then incubated in 25 µl of FSB containing 20 µg/ml of streptavidin-FITC conjugate (Southern Biotechnology Associates, Inc.) for 30 minutes on ice in the dark. The cells were washed once with 4 ml of FSB, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to human β 2m using a FACScan™ flow cytometer (BD® Biosciences, San Jose, Calif.). Several clones with the highest apparent staining were subcloned using a FACStar cell sorter (BD® Biosciences), expanded in DMEM, 10% FBS, 2 mM L-glutamine, and retested by FACSTM as described above. One subclone, designated NS0 HuFcRn (memb), clone 7-3, was used in subsequent binding assays.

Please replace the paragraph at page 66, lines 2-14, with the following amended paragraph:

Concentrated OST577-IgG2M3 supernatants were tested in a single-point competitive binding assay for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3. Approximately 2×10^5 cells/test were washed once in FACS Binding Buffer (FBB) (PBS containing 0.5% BSA, 0.1% NaN₃), pH 8.0, once in FBB, pH 6.0, and resuspended in 120 µl of pre-mixed biotinylated OST577-IgG2M3 antibody (8.3 µg/ml) and concentrated supernatant (containing 8.3 µg/ml of competitor antibody) in FBB, pH 6.0. The cells were incubated for 1 hour on ice, washed twice in FBB, pH 6.0, and resuspended in 25 µl of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 µg/ml in FBB, pH 6.0. After incubation for 30 minutes

on ice in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) of each mutant was compared to that of the wild-type antibody and plotted using Excel (Microsoft® Corporation, Redmond, Wash.).

Please replace the paragraph at page 66, lines 16-30, with the following amended paragraph:

A dilution series of each purified OST577-IgG2M3 antibody was competed against biotinylated HuEP5C7-IgG2M3 antibody (He et al., J. Immunol. 160:1029-1035 (1998)) for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3. For initial screening experiments, approximately 2×10^5 cells/test were washed once in FSB, pH 6.0, and resuspended in 100 μ l of pre-mixed biotinylated HuEP5C7-IgG2M3 antibody (10 μ g/ml) and OST577-IgG2M3 competitor antibody (twofold serial dilutions from 208 μ g/ml to 0.102 μ g/ml) in FSB, pH 6.0. The cells were incubated with the antibody mixture for 1 hour on ice, washed twice in FSB, pH 6.0, and resuspended in 25 μ l of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 μ g/ml in FSB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FSB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACScan™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) was plotted against competitor concentration, and IC50 values were calculated using GraphPad Prism® (GraphPad™ Software, Inc., San Diego, Calif.). For consistency, the IC50 values shown in the Tables are based on the final competitor concentrations.

Please replace the paragraph at page 67, lines 12-26, with the following amended paragraph:

A dilution series of each purified OST577-IgG2M3 antibody was competed against biotinylated OST577-IgG2M3 antibody for binding to rhesus FcRn on cell line NS0 RhFcRn, clone R-3. In one group of experiments, approximately 2×10^5 cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 120 μ l of pre-mixed biotinylated OST577-IgG2M3 antibody (8.3 μ g/ml) and OST577-IgG2M3 competitor antibody (twofold serial dilutions from 208 μ g/ml to 0.102 μ g/ml) in FBB, pH 6.0. The cells were incubated with the antibody mixture for 1 hour on ice, washed twice in FBB, pH 6.0, and resuspended in 25 μ l of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 μ g/ml in FBB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences). Another group of experiments was done in 200 μ l of pre-mixed biotinylated OST577-IgG1 antibody (5.0 μ g/ml) and OST577-IgG1 competitor antibody (threefold serial dilutions starting from 500 μ g/ml) in FBB, pH 6.0, as described above.

Please replace the paragraph beginning at page 73, lines 4-14, with the following amended paragraph:

Purified OST577-IgG2M3 antibodies were tested for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3, or to untransfected NS0 cells in FBB at pH 6.0. Approximately 2×10^5 cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 100 μ l of antibody at a concentration of 11 μ g/ml in FBB, pH 6.0. The cells were incubated with antibody for 1 hour on ice, washed twice in FBB, pH 6.0, and resuspended in 25 μ l of goat anti-human IgG RPE-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 5 μ g/ml in FBB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACScan™ flow cytometer (BD®

Biosciences). Mean channel fluorescence (MCF) of each mutant was plotted using Excel (Microsoft® Corporation).

Please replace the paragraph at page 73, lines 14-29, with the following amended paragraph:

A dilution series of each purified OST577-IgG2M3 antibody was competed against biotinylated OST577-IgG2M3 antibody for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3 at 37° C. Approximately 2×10^5 cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 100 μ l of pre-mixed biotinylated OST577-IgG2M3 antibody (10 μ g/ml) and OST577-IgG2M3 competitor antibody (twofold serial dilutions, from 208 μ g/ml to 0.102 μ g/ml) in FBB, pH 6.0. The cells were incubated with the antibody mixture for 1 hour at 37° C., washed twice in FBB, pH 6.0, and resuspended in 25 μ l of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 μ g/ml in FBB, pH 6.0. After incubation for 30 minutes in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACScan™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) was plotted against competitor concentration, and IC50 values were calculated using GraphPad Prism® (GraphPad™ Software).

Please replace the paragraph beginning at page 73, line 31, with the following amended paragraph:

Purified OST577-IgG2M3 and OST577-IgG1 mutant antibodies were compared to the respective wild-type antibodies for binding to human FcRn and then released at various pH values in single-point binding and release assays using cell line NS0 HuFcRn (memb), clone 7-3. Approximately 2×10^5 cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 100 μ l of purified antibody (10 μ g/ml) in FBB, pH 6.0. The cells were

incubated for 1 hour on ice, washed twice in FBB, pH 6.0, 6.5, 7.0, 7.5, or 8.0, and resuspended in 25 μ l of goat F(ab')₂ anti-human IgG FITC-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 1.25 μ g/ml in FBB of the appropriate pH. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB of the appropriate pH, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACSTM using a FACSCaliburTM flow cytometer (BD[®] Biosciences). Mean channel fluorescence (MCF) of each mutant was plotted using Excel (Microsoft[®] Corporation).

Please replace the paragraph beginning at page 76, line 28, with the following amended paragraph:

The antigen binding activity of Hu1D10-IgG2M3 wild-type and mutant antibodies was confirmed in a FACSTM binding assay using Raji cells, which express an allele of the HLA-DR β chain that is recognized by Hu1D10 (Kostelný et al. (2001), op. cit.). Approximately 2.5×10^5 cells/test were washed once in FBB, pH 7.4, and resuspended in 140 μ l of Hu1D10-IgG2M3 antibody (threefold serial dilutions from 60 μ g/ml to 0.027 μ g/ml) in FBB, pH 7.4. The cells were incubated with antibody for 1 hour on ice, washed twice in FBB, pH 7.4, and resuspended in 25 μ l of goat F(ab')₂ anti-human kappa RPE-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 10 μ g/ml in FBB, pH 7.4. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 7.4, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to the HLA-DR β chain allele by FACSTM using a FACSCaliburTM flow cytometer (BD[®] Biosciences).

Please replace the paragraph at page 77, lines 7-19, with the following amended paragraph:

Similarly, the antigen binding activity of Hu1D10-IgG1 wild-type and mutant antibodies was confirmed in a FACSTM binding assay using Raji cells. Approximately 2.0×10^5 cells/test

were washed once in FBB, pH 7.4, and resuspended in 100 μ l of Hu1D10-IgG1 antibody (twofold serial dilutions from 25 μ g/ml to 12.5 μ g/ml, then threefold serial dilutions from 12.5 μ g/ml to 0.0020 μ g/ml) in FBB, pH 7.4. A dilution series of HuFd79-IgG1 antibody (Co et al., Proc. Natl. Acad. Sci. 88:2869-2873 (1991)) was prepared as described above and used as a negative control. The cells were incubated with antibody for 1 hour on ice, washed twice in FBB, pH 7.4, and resuspended in 25 μ l of goat F(ab')₂ anti-human IgG FITC-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 20 μ g/ml in FBB, pH 7.4. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 7.4, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to the HLA-DR β chain allele by FACSTM using a FACSCaliburTM flow cytometer (BD® Biosciences).